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Isolation of cells from the epithelial layer of frozen human intestinal biopsies

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We use this protocol and it's working

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Abstract

This protocol is for the isolation of the epithelial cells (and associated immune cells within the epithelial layer) from frozen human intestinal pinch biopsies, for use in single-cell sequencing or flow cytometry experiments. This protocol is adapted from Parikh, Antanaviciute, Fawkner-Corbett et al., (2019) Natuer (doi: 10.1038/s41586-019-0992-y). It has been modified specifically with the aim of increasing cell recovery from biopsies of the small intestine.

Attachments

PDF

HCA Epithelial cell ...

70KB

Materials

- **Transport medium [500ml**] – 490ml DMEM (high glucose), 5ml Pen/Strep, 5ml HEPES (stock = 1M)
- **T5 medium [50ml]** – 47.5ml of Transport Media + 2.5ml FCS
- **HPGA [500ml]** – 500ml HBSS, 5ml Pen/Strep, 5ml HEPES (1M)
- **Wash medium [50ml]** – 50ml HPGA + 1mM EDTA (100ul of 0.5M in 50ml) + 1mM DTT (reconstitute in 100ul of ddH_2O to make 500mM solution, add to 50ml medium to make 1mM)
- **Chelation medium [50ml]** – 50ml HPGA + 1mM EDTA (100ul of 0.5M in 50ml)

Troubleshooting

Warming media

1 Per sample: pre-warm 10ml of Transport medium in the waterbath.

2 Per sample: pre-warm 20ml of chelation medium in the waterbath.

Collection/Wash

3 Partially thaw frozen biopsies in 37°C waterbath until only a little ice remains.

4 Dropwise add 1ml of pre-warmed transport media to the sample to thaw completely.

5 Use a pastette to transfer the biopsies into the conical of pre-warmed transport media.

6 Place a 70 micron filter onto a 50ml conical.

7 Transfer biopsies onto the filter.

8 Wash with 20ml of cold wash medium.

Crypt isolation

9 Transfer biopsies to 5ml warm chelation medium (in 50ml conical).

10 Incubate at 37°C for 10 mins (in incubator).

11 Vortex 2 × 5-10s.

12 Allow biopsies to settle. Remove biopsies from the chelation medium (containing potential isolated crypts) with FCS-coated pastette and transfer to fresh Falcon with 5ml warm chelation medium. Return the biopsies in fresh medium to 37°C incubator and repeat steps 10-12.

13 Store chelation medium with the isolated crypts at 4°C while proceeding with subsequent isolation steps.

14 Repeat steps 9-13 for a total of 4 times, and pool crypt containing fractions.

Single cell suspention from isolated crypts

15 Spin down the pooled crypt suspension (300 g, 5 mins).

16 Resuspend in 3 ml warm TrypLE Express with 50 µg/ml DNAse at 37°C (75 µl of 2 mg/ml stock).

17 Incubate 60 mins at 37°C in incubator shaking incubator (220 RPM).

18 Filter with 70 µm cell strainer (prepped with FCS) into 50 ml Falcon, wash through with 5 ml of T5 media.

19 Spin filtrate 1500 RPM 5mins.

20 Wash pellet with 30ml T5 media.

21 Spin 1500 RPM 5mins.

22 Very carefully remove supernatant until less than 1ml remains. Resuspend pellet in this.

23 Run through 30um cell strainer (prepped with FCS) and wash through with 5ml of PBS.

24 Spin 1500 RPM 5 mins, re-suspend in approximately 1ml of remaining PBS, transfer to Eppendorf.

25 Make up to 1ml with PBS and take 10 uL for count (1:1 with Trypan blue).

26 For 10X single-cell sequencing, count each sample twice using countess to obtain average count and viability:

27 Spin down in micro centrifuge (1500 RPM, 5 mins) and re-suspend using live count to concentration of 2000 cells/µl (2×10^6 cells/ml).

28 re-check count and make volume to 1500 cells/µl (1.5×10^6 cells/ml) with PBS.

29 Transfer to single cell facility on ice.

Flow cytometry to confirm cell recovery

30 Transfer epithelial cells to a round bottom 96 well plate.

31 Spin plate at 2200RPM for 2 mins, and dispose of supernatant.

32 Add 200 µl wash buffer, and spin at 2200 RPM for 1min **x2.**

33 Remove supernatant and add 100 µl of FACS buffer.

34 Add 1:100 EpCAM-PE (1 µl), 1:50 CD24-PE-CF594 (2 µl) and 1:100 CD45-AF700 (1 µl).

35 Place in fridge for 20 mins.

36 Spin plate at 2200 RPM for 1 min, and dispose of supernatant.

37 Add 200µl wash buffer, and spin at 2200 RPM for 1min **x2.**

38 Resuspend in 300µl of FACS buffer and add 1:100 (3 µl) of diluted Sytox Green.

39 a.1:60 (5 µl Sytox Green + 295 µl FACS buffer) – final dilution 1:6000.

40 10.Transfer to tubes and run through FACS.